

CLAIMS:

1. A mutant castor  $\Delta^9$ -18:0-ACP desaturase having one or more amino acid substitutions selected from the group consisting of:

- a) Ala or Thr for Met at residue 114 of SEQ ID NO: 1;
- b) Arg for Thr at residue 117 of SEQ ID NO: 1;
- c) Gly or Ala for Leu at residue 118 of SEQ ID NO: 1;
- d) Val or Leu for Pro at residue 179 of SEQ ID NO: 1;
- e) Val or Ser for Thr at residue 181 of SEQ ID NO: 1; and
- f) Leu for Gly at residue 188 of SEQ ID NO: 1.

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2. The mutant castor  $\Delta^9$ -18:0-ACP desaturase of Claim 1 which has the amino acid substitution Arg for Thr at residue 117 of SEQ ID NO: 1.

3. The mutant castor  $\Delta^9$ -18:0-ACP desaturase of Claim 1 which has the amino acid substitution Arg for Thr at

residue 117 of SEQ ID NO: 1 and Leu for Gly at residue 188 of SEQ ID NO: 1.

4. The mutant castor  $\Delta^9$ -18:0-ACP desaturase of Claim 1 which contains each of the following amino acid substitutions:

- a) Ala for Met at residue 114 of SEQ ID NO: 1;
- b) Arg for Thr at residue 117 of SEQ ID NO: 1;
- c) Gly for Leu at residue 118 of SEQ ID NO: 1;
- d) Val for Pro at residue 179 of SEQ ID NO: 1;
- e) Val for Thr at residue 181 of SEQ ID NO: 1;
- and
- f) Leu for Gly at residue 188 of SEQ ID NO: 1.

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5. The mutant castor  $\Delta^9$ -18:0-ACP desaturase of Claim 1 which contains each of the following amino acid substitutions:

- a) Thr for Met at residue 114 of SEQ ID NO: 1;
- b) Arg for Thr at residue 117 of SEQ ID NO: 1;
- c) Ala for Leu at residue 118 of SEQ ID NO: 1;
- d) Leu for Pro at residue 179 of SEQ ID NO: 1;
- e) Ser for Thr at residue 181 of SEQ ID NO: 1;
- and
- f) Leu for Gly at residue 188 of SEQ ID NO: 1.

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6. A DNA expression construct comprising, in expressible form, a nucleic acid sequence which encodes a

mutant castor  $\Delta^9$ -18:0-ACP desaturase having one or more amino acid substitutions selected from the group consisting of:

- a) Ala or Thr for Met at residue 114 of SEQ ID NO: 1;
- b) Arg for Thr at residue 117 of SEQ ID NO: 1;
- c) Gly or Ala for Leu at residue 118 of SEQ ID NO: 1;
- d) Val or Leu for Pro at residue 179 of SEQ ID NO: 1;
- e) Val or Ser for Thr at residue 181 of SEQ ID NO: 1; and
- f) Leu for Gly at residue 188 of SEQ ID NO: 1.

7. A DNA expression construct comprising, in expressible form, a nucleic acid sequence which encodes a mutant castor  $\Delta^9$ -18:0-ACP desaturase having each of the following amino acid substitutions:

- a) Ala for Met at residue 114 of SEQ ID NO: 1;
- b) Arg for Thr at residue 117 of SEQ ID NO: 1;
- c) Gly for Leu at residue 118 of SEQ ID NO: 1;
- d) Val for Pro at residue 179 of SEQ ID NO: 1;
- e) Val for Thr at residue 181 of SEQ ID NO: 1;
- and
- f) Leu for Gly at residue 188 of SEQ ID NO: 1.

8. A DNA expression construct comprising, in expressible form, a nucleic acid sequence which encodes a mutant castor  $\Delta^9$ -18:0-ACP desaturase having each of the following amino acid substitutions:

- a) Thr for Met at residue 114 of SEQ ID NO: 1;
- b) Arg for Thr at residue 117 of SEQ ID NO: 1;
- c) Ala for Leu at residue 118 of SEQ ID NO: 1;
- d) Leu for Pro at residue 179 of SEQ ID NO: 1;
- e) Ser for Thr at residue 181 of SEQ ID NO: 1;
- and
- f) Leu for Gly at residue 188 of SEQ ID NO: 1.

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9. A cell transformed with the DNA expression construct comprising, in expressible form, a nucleic acid sequence which encodes a mutant castor  $\Delta^9$ -18:0-ACP desaturase having one or more amino acid substitutions selected from the group consisting of:

- a) Ala or Thr for Met at residue 114 of SEQ ID NO: 1;
- b) Arg for Thr at residue 117 of SEQ ID NO: 1;
- c) Gly or Ala for Leu at residue 118 of SEQ ID NO: 1;
- d) Val or Leu for Pro at residue 179 of SEQ ID NO: 1;

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e) Val or Ser for Thr at residue 181 of SEQ ID NO: 1; and

f) Leu for Gly at residue 188 of SEQ ID NO: 1.

10. The cell of Claim 9 which is a prokaryotic cell.

11. The cell of Claim 9 which is an eukaryotic cell.

12. The cell of Claim 11 which is a plant cell.

13. A transgenic plant expressing a nucleic acid sequence which encodes a mutant castor  $\Delta^9$ -18:0-ACP desaturase having one or more amino acid substitutions selected from the group consisting of:

a) Ala or Thr for Met at residue 114 of SEQ ID NO: 1;

b) Arg for Thr at residue 117 of SEQ ID NO: 1;

c) Gly or Ala for Leu at residue 118 of SEQ ID NO: 1;

d) Val or Leu for Pro at residue 179 of SEQ ID NO: 1;

e) Val or Ser for Thr at residue 181 of SEQ ID NO: 1; and

f) Leu for Gly at residue 188 of SEQ ID NO: 1.

14. The transgenic plant of Claim 13 which is *Arabidopsis thaliana*.

15. A transgenic plant expressing a nucleic acid sequence which encodes a mutant castor  $\Delta^9$ -18:0-ACP

desaturase having each of the following amino acid substitutions:

- a) Ala for Met at residue 114 of SEQ ID NO: 1;
- b) Arg for Thr at residue 117 of SEQ ID NO: 1;
- c) Gly for Leu at residue 118 of SEQ ID NO: 1;
- d) Val for Pro at residue 179 of SEQ ID NO: 1;
- e) Val for Thr at residue 181 of SEQ ID NO: 1;
- and
- f) Leu for Gly at residue 188 of SEQ ID NO: 1.

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16. The transgenic plant of Claim 15 which is *Arabidopsis thaliana*.

17. A transgenic plant expressing a nucleic acid sequence which encodes a mutant castor  $\Delta^9$ -18:0-ACP desaturase having each of the following amino acid substitutions:

- a) Thr for Met at residue 114 of SEQ ID NO: 1;
- b) Arg for Thr at residue 117 of SEQ ID NO: 1;
- c) Ala for Leu at residue 118 of SEQ ID NO: 1;
- d) Leu for Pro at residue 179 of SEQ ID NO: 1;
- e) Ser for Thr at residue 181 of SEQ ID NO: 1;
- and
- f) Leu for Gly at residue 188 of SEQ ID NO: 1.

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18. The transgenic plant of Claim 17 which is *Arabidopsis thaliana*.

19. A DNA expression construct comprising, in expressible form, a nucleic acid sequence which encodes a mutant castor  $\Delta^9$ -18:0-ACP desaturase which has the amino acid substitution Arg for Thr at residue 117 of SEQ ID NO: 1.

20. A cell transformed with a DNA expression construct comprising, in expressible form, a nucleic acid sequence which encodes a mutant castor  $\Delta^9$ -18:0-ACP desaturase which has the amino acid substitution Arg for Thr at residue 117.

21. The cell of Claim 20 which is prokaryotic.

22. The cell of Claim 20 which is eukaryotic.

23. The cell of Claim 22 which is a plant cell.

24. A transgenic plant expressing a nucleic acid sequence which encodes a mutant castor  $\Delta^9$ -18:0-ACP desaturase which has the amino acid substitution Arg for Thr at residue 117.

25. The transgenic plant of Claim 24 which is *Arabidopsis thaliana*.

26. A cell transformed with a DNA expression construct comprising, in expressible form, a nucleic acid sequence which encodes a mutant castor  $\Delta^9$ -18:0-ACP desaturase which contains each of the following amino acid substitutions:

a) Ala for Met at residue 114;

- 10           b)    Arg for Thr at residue 117;  
             c)    Gly for Leu at residue 118;  
             d)    Val for Pro at residue 179;  
             e)    Val for Thr at residue 181; and  
             f)    Leu for Gly at residue 188.

27.   The cell of Claim 26 which is prokaryotic.

28.   The cell of Claim 26 which is eukaryotic.

29.   The cell of Claim 28 which is a plant cell.

30.   A mutant castor  $\Delta^9$ -18:0-ACP desaturase which has an amino acid substitution of Phe for Thr at residue 181 of SEQ ID NO: 1.

31.   A mutant castor  $\Delta^9$ -18:0-ACP desaturase which has the amino acid substitution Trp for Thr at residue 181 of SEQ ID NO: 1.

32.   A DNA expression construct comprising, in expressible form, a nucleic acid sequence which encodes a mutant castor  $\Delta^9$ -18:0-ACP desaturase which has an amino acid substitution of Phe for Thr at residue 181 of SEQ ID NO: 1.

33.   A DNA expression construct comprising, in expressible form, a nucleic acid sequence which encodes a mutant castor  $\Delta^9$ -18:0-ACP desaturase which has an amino acid substitution of Trp for Thr at residue 181 of SEQ ID NO: 1.



34. A cell transformed with a DNA expression construct comprising, in expressible form, a nucleic acid sequence which encodes a mutant castor  $\Delta^9$ -18:0-ACP desaturase which has an amino acid substitution of Phe for Thr at residue 181 of SEQ ID NO: 1.

35. The cell of Claim 34 which is a prokaryotic cell.

36. The cell of Claim 34 which is a eukaryotic cell.

37. The cell of Claim 36 which is a plant cell.

38. A transgenic plant expressing a nucleic acid sequence which encodes a mutant castor  $\Delta^9$ -18:0-ACP desaturase which has an amino acid substitution of Phe for Thr at residue 181 of SEQ ID NO: 1.

39. The transgenic plant of Claim 38 which is *Arabidopsis thaliana*.

40. A method for specifically altering a function of a protein through directed mutagenesis, comprising:

- a) identifying candidate amino acid positions of the protein which when mutated are predicted to alter the function;
- b) generating a library of mutants of the protein, the mutants being generated by randomization of the amino acid encoded at each candidate position, in combination with

- 10 randomization of every other candidate position;  
and  
c) identifying mutants which exhibit the desired  
specific alteration of function from the library  
of mutants.

41. The method of Claim 40 wherein the candidate amino acid positions are identified by a combination of methods.

42. The method of Claim 40 wherein the candidate positions comprise positions of amino acids which directly participate in the function which is to be altered.

43. The method of Claim 42 wherein the candidate positions further comprise positions of amino acids which indirectly participate in the function which is to be altered.

44. The method of Claim 40 wherein the candidate positions are identified by random mutagenesis.

45. The method of Claim 40 wherein the candidate positions are identified by structural analysis of the protein.

46. The method of Claim 40 wherein the candidate positions are identified by sequence analysis and comparison to related proteins.

47. The method of Claim 40 wherein the library of mutants is generated by overlap extension PCR.

48. The method of Claim 40 wherein mutants which exhibit the desired alteration of function are identified by a selective screening process.

49. The method of Claim 40 wherein the protein is an enzyme.

50. The method of Claim 49 wherein the enzyme is castor  $\Delta^9$ -18:0-ACP desaturase.

51. The method of Claim 49 wherein substrate specificity of the enzyme is altered.

52. The method of Claim 49 wherein *in vivo* activity of the enzyme is altered.

53. The method of Claim 49 wherein *in vitro* activity of the enzyme is altered.

54. The method of Claim 49 wherein *in vivo* and *in vitro* activity of the enzyme is altered.

55. The method of Claim 40 wherein the protein is a ligand binding protein.

56. The method of Claim 55 wherein the *in vivo* ligand binding specificity of the protein is altered.

57. The method of Claim 55 wherein the *in vitro* ligand binding specificity of the protein is altered.

58. The method of Claim 55 wherein the *in vivo* and *in vitro* ligand binding specificity of the protein are altered.

